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INSECTICIDAL ACTIVITIES OF FIVE MEDICINAL PLANT MATERIALS AGAINST CALLOSOBRUCHUS MACULATUS FABRICIUS (COLEOPTERA: CHRYSOMELIDAE) INFESTING COWPEA SEEDS IN STORAGE

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ABSTRACT

Powders from five elite plant materials (nutmeg, *Myristica fragrans*; red hot chilli pepper, *Capsicum annuum*; ginger rhizome, *Zingiber officinale*; black pepper, *Piper nigrum* and alligator pepper, *Aframomum melegueta*) at 1.5 % w/w concentration were assessed against cowpea bruchid, *Callosobruchus maculatus* infestations on stored cowpea seeds under laboratory environmental conditions in three agro-ecological zones in Nigeria by members of the Research Group. The conventional synthetic insecticide, permethrin was included as standard check, while untreated seeds served as the control. Adult mortality, oviposition rate and adult emergence were the indices considered in the experiments. For the first three days of exposure, black pepper (*P. nigrum*) powder caused the highest percentage mortality (68.8, 78.0 and 83.8 % respectively) of the bruchid adults and differed significantly ($P < 0.01$) from the rest of other treatments with the exception of permethrin that caused 78.8, 85.0 and 91.3 % mortality respectively. Similarly, black pepper powder and permethrin treatments differed significantly ($P < 0.01$) from the rest on number of eggs laid and emerged adults with the exception of *Z. officinale* rhizome, which did not differ significantly from *P. nigrum* on number of eggs laid. The results therefore suggest that black pepper powder has higher insecticidal potential over other plant materials and could serve as a biotechnological substitute to the synthetic insecticides in the control of *C. maculatus* infestations and damage in stored products.

Contribution/Originality: The paper's primary contribution is finding that some bioinsecticides derived from medicinal plant materials could serve as substitute to the hazardous synthetic insecticides at economically justified concentration in the control of C. maculatus infestation and damage in stored cowpea seeds.

1. INTRODUCTION

Cowpea, *Vigna unguiculuta* (L. Walp) is an important food crop in tropical countries particularly in West Africa (Adedire and Ajayi, 2003). It has been reported that the crop is also a source of livestock feed and revenue in the tropics (Onekutu *et al*., 2015). Nigeria contributes a substantial amount of world cowpea production with about four million hectares cultivated annually (Ofuya and Longe, 2006). Cowpea seed is a main source of plant proteins for the families that consume it in many countries of Western and Central Africa (Ileke *et al*., 2012).

Callosobruchus maculatus is a principal pest of stored legume seeds, particularly cowpea, *Vigna unguiculata* (Ofuya, 2001). *C. maculatus* infestations have been reported to cause substantial reduction in quality and quantity of cowpea seeds -within three to five months of storage (Ileke *et al*., 2012). Furthermore, Ashamo (2007) opined that the biggest challenge facing Nigerian farmers is that of post-harvest losses associated with insect pest infestation. This is because preservation of the cowpea seeds for the next cropping season is a major target of the farmer.

Currently, synthetic insecticides application is the major means of controlling beetles infestations in stored cowpea seeds (Onekutu *et al*., 2015). This could be in form of fumigation of stored product with phosphine or carbon disulphide and or dusting with carbaryl, pirimiphos methyl or permethrin (Ileke *et al*., 2012). However, consequent upon reported ozone depletion by methyl bromide and carcinogenic concerns with phosphine, conventional fumigation technology is under scrutiny in the developed countries (Adedire *et al*., 2011). Ileke *et al*. (2012) further highlighted problems associated with the use of conventional synthetic insecticides to include high mammalian toxicity, high level of persistence in the environment, poor application knowledge, exorbitant cost prices, pest resurgence, genetic resistance by the insect pest and deleterious effects on non-target organisms. One possible way to overcome the short comings of synthetic insecticides is to substitute synthetic insecticides with naturally-occurring plant insecticidal materials (Ileke *et al*., 2012; Khater, 2012). According to Lale (1995) plant products are neither scarce nor expensive; they are safe for humans and environmentally safer than the conventional synthetic insecticides. Since the control of this coleopteran insect pest with synthetic insecticides has been reported to be unsafe to humans (Ileke *et al*., 2012; Onekutu *et al*., 2015) the present study sought to assess powders of five medicinal plant materials which are peppers and spices against *C. maculatus* infestation on stored cowpea seeds in three different agro-ecological zones of Nigeria.

2. MATERIALS AND METHODS

2.1. Standardization of Seeds

Cowpea variety TVX3236 used in this study was obtained from the Seed Technology Centre, Federal University of Agriculture Makurdi, Benue State, Nigeria. The seeds were cleaned and frozen at -20 ± 2°C for one week to disinfest them and then, stored at 4°C to prevent re-infestation (Sulehrie *et al*., 2003). Prior to the bioassay, cowpea seeds used for the experiment were kept in muslin-covered plastic containers to acclimatize for four weeks. As measured by the standard oven method, this stabilized the moisture contents of the seeds at about 14 % under laboratory mean temperature and relative humidity of 31.2 °C and 69.8 % respectively.

2.2. Mass Rearing of Callosobruchus maculatus

Callosobruchus maculatus adults were obtained from the stock culture of the Department of Crop and Environmental Protection, Federal University of Agriculture Makurdi, Benue State, Nigeria. The insects were reared on a susceptible cowpea variety, Ife brown in 1.5-litre glass bottles at laboratory ambient temperature and relative humidity of 31.2 °C and 69.8 % respectively. Freshly emerged bruchid adults of 1 - 2 days old were then used for the experiment.

2.3. Plant Materials

The five medicinal plant materials and their identities used in the investigation are presented in Table 1. Air dried samples of the plant materials were bought from spice sellers at Modern Market, Makurdi, Benue State, Nigeria. The plant materials were ground separately into powder using clean mortar and pestle. The powders were sieved (mesh size: 1 mm^2) to produce fine powders (Ileke and Oni, 2011) which were used immediately for the experiment. The choice of "test concentration" of plant powder was based on Lale (2002) which stressed that the concentration of plant powder used against stored product pests should not be greater than 2.0 % w/w to be economically-justified. Therefore, the plant materials were assessed at 1.5 % w/w concentration.

Table-1. Plants evaluated for insecticidal properties against *C. maculatus* Fab. in stored cowpea seeds

Scientific name	Common name	Family	Part used	Availability	Places found
Myristica fragrans	Nutmeg	Myristicaceae	Nut	Readily available	Market, farm
Capsicum annuum	Red hot chilli pepper	Solanaceae	Fruit	Readily available	Market, farm
Zingiber officinale	Ginger	Zingiberaceae	Rhizome	Readily available	Market, farm
Piper nigrum	Black pepper	Piperaceae	Seed	Readily available	Market, farm
Aframomum melegueta	Alligator pepper	Zingiberaceae	Seed	Readily available	Market, farm

2.4. Bioassay

A quantity of 0.3 g powder of each of the plant materials: *M. fragrans*, *C. annuum*, *Z. officinale*, *P. nigrum* and *A. melegueta* was added respectively to five plastic jars (each of 100 ml capacity) containing 20 g of cowpea seeds (TVX3236) to give 1.5 % w/w. Comparable rate of permethrin (Rambo: 0.6 % D) was added into a sixth jar containing 20 g of cowpea seeds. The plant powders and seeds were mixed thoroughly to enhance coating of the seeds. An untreated control was also set up with no plant material powder and permethrin added. Five pairs (5 females + 5 males) of freshly-emerged adult *C. maculatus* were introduced into each jar which was covered with a muslin cloth and held firmly with a rubber band. Female and male *C. maculatus* adults were sexed using the method of Raina (1970). Adults with shorter antennae and bright-spotted elytra were identified as female while those having longer antennae and dull-coloured elytra were characterized as male. The treatments were arranged in a completely randomized design on a workbench in the laboratory. The experiment was observed daily for 4 days and data on percentage adult bruchid mortality were collected every 24 hours and recorded. A bruchid was considered dead if it did not respond to a probe with a pin. Percent mortality was calculated using the standard formula:

$$\frac{Number\ of\ dead\ C.maculatus\ adults\ X\ 100}{Total\ number\ of\ C.maculatus\ adults}$$

All dead and live *C. maculatus* adults were sieved out immediately after mortality count to ensure that the emerging adults were direct consequence of the number of eggs oviposited in 4 days (Ileke *et al.*, 2012). The eggs laid on the cowpea seeds after 96 hours in a no-choice protocol were counted and the average number was calculated per treatment. The examined seeds were returned to their respective containers and left under the same experimental conditions for another 26 days, giving a total of 30 days under storage and emerged adults from seeds of different treatments were counted and recorded. The experiment was replicated four times.

3. STATISTICAL ANALYSIS

The statistical software was SPSS for Windows® (version 21.0). Data were subjected to one - way analysis of variance (ANOVA). Following the outcome of Levene's test for equality of variances, data were transformed using arcsine and log N transformations, respectively (Somta *et al.*, 2008). Tukey's Honest Significant Difference (HSD) test was used to separate significant means from non - significant ones at $\alpha = 0.05$.

4. RESULTS

4.1. Effect of the Plant Materials on Mortality of Adult C. maculatus

Effect of the plant materials on adult mortality of *C. maculatus* is presented in Table 2. Percent mortality varied significantly ($P < 0.01$) among the treatments. All the test materials caused mortality in various degrees to the beetle after 24 hours of exposure, reaching 100 % in 96 hours. *Piper nigrum* caused highest bruchid mortality at all exposure periods and this differed significantly from mortalities caused by the rest of the plant materials. The conventional synthetic insecticide, permethrin, caused higher bruchid mortality than *P. nigrum* at 24 - hour post - treatment period. However, at 2nd to 4th day exposure periods, the efficacy of *P. nigrum* matched permethrin in the mortality of the adult caused. Significant differences did not occur among *M. fragrans*, *C. annuum* and untreated control in terms of beetle mortality after 24 hours of exposure. The *A. melegueta* seed powder did not cause considerable *C. maculatus* mortality within 48 hours of exposure. However, powder of *C. annuum* which was the least active killed all the beetles after 96 hours as recorded for other treatments.

4.2. Effect of the Plant Materials on Oviposition and Adult Emergence of C. maculatus

Table 3 presents results on oviposition deterrence by the plant materials caused to *C. maculatus* females as well as effects of the plant materials on adult emergence. The number of eggs laid by female *C. maculatus* adults was also significantly different (P < 0.01) among treatments. *Piper nigrum* deterred oviposition most. This was followed by *Z. officinale*. *Capsicum annum* gave the highest support to oviposition. However, no plant material matched the efficacy of permethrin in deterring oviposition in the bruchids. Adult emergence was significantly lower in cowpea seeds protected with powder of *P. nigrum* and highest in seeds protected with powder of *C. annum*. *Piper nigrum* matched permethrin in reducing adult emergence. *Aframomum melegueta* deterred oviposition and reduced adult emergence significantly when compared to the unprotected cowpea seeds; but not when compared to permethrin. Cowpea seeds treated with *M. fragrans*, *Z. officinale* and *A. melegueta* did not differ significantly in the number of emerged adults.

Table-2. Effect of plant materials [1.5 % (w/w)] and permethrin powder on mortality of adult *C. maculatus* Fab.

Treatment	% mortality at indicated hour				Performance rank
	24	48	72	96	
Myristica fragrans	10.00±0.20[a]	20.00±0.220[a]	30.00±0.13[a]	100.00±0.00[a]	4th
Capsicum annuum	8.80±0.32[a]	9.30±0.53[b]	20.00±0.04[a]	100.00±0.00[a]	5th
Zingiber officinale	43.00±1.00[b]	55.00±0.87[c]	63.00±3.21[b]	100.00±0.00[a]	2nd
Piper nigrum	68.80±0.04[c]	78.00±0.32[d]	83.80±0.45[c]	100.00±0.00[a]	1st
Aframomum melegueta	28.00±0.20[d]	43.80±6.30[c]	51.30±1.00[b]	100.00±0.00[a]	3rd
Permethrin powder*	78.80±0.32[c]	85.00±0.00[d]	91.30±0.34[c]	100.00±0.00[a]	Standard insecticide
Untreated control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	Unprotected cowpea

*Treated control (conventional synthetic insecticide)
Data are means ± SEM of four replications
Means in a column followed by the same letter are not significantly different (α = 0.05)
Mortality was significant at P < 0.01 by Fisher's F-test

Table-3. Effect of plant materials [1.5 % (w/w)] and permethrin powder on oviposition and adult emergence by *C. maculatus* Fab.

Protectants	No. of eggs laid (n/ 20 g cowpea seeds) **	No. of emerged adults***
Myristica fragrans	62.70±0.48[a]	57.00±0.91[a]
Capsicum annuum	82.50±2.75[b]	76.30±0.04[d]
Zingiber officinale	46.00±0.09[c]	44.00±5.01[a]
Piper nigrum	35.00±0.28[c]	32.30±8.03[c]
Aframomum melegueta	55.25±0.01[a]	54.00±0.52[a]
Permethrin powder*	17.00±0.06[d]	20.30±7.23[c]
Untreated control	145.50±0.45[e]	145.00±0.77[d]

*Treated control (conventional synthetic insecticide)
**After 4 days
***After 30 days
Data are means ± SEM of four replications
Means in a column followed by the same letter is not significantly different (α = 0.05)
All indices were significant at P < 0.01 by Fisher's F-test

5. DISCUSSION

This investigation revealed that powder of *P. nigrum* had the highest insecticidal activity against *C. maculatus*. Its toxic effect on the insect did not significantly differ from permethrin in 24 hours. Adult emergence deterrence by *P. nigrum* also showed that the black pepper has good potentials for controlling bruchid infestations in stored cowpea seeds. This agrees with the findings that members of the family Piperaceae show some form of insecticidal activity (Adedire and Ajayi, 1996; Okonkwo and Okoye, 1996; Adedire and Lajide, 1999). Okonkwo and Okoye (1996) reported that the bioactive agents in some members of Piperaceae are piperine and chavicine and these are highly insecticidal to various crop pests. Lale (1995) reported piperine and alkaloids as the major active ingredients in *P. guineense* seeds. This can be attributed to the reason why *P. nigrum* was significantly better at evoking insecticidal activity. However, the results presented here indicate that the conventional synthetic insecticide permethrin was superior to *P. nigrum* on account of the latter lacking strong evidence of oviposition deterrence.

The failure of *A. melegueta* seed powder to cause considerable *C. maculatus* mortality within 48 hours of exposure corroborates the findings of Adedire and Lajide (1999) and Onekutu *et al.* (2015) it has been reported that both seed powder and extract of *A. melegueta* did not affect oviposition and egg hatchability of *C. maculatus* significantly (Ofuya, 1990). Differences in mortality data of untreated control and mortality caused by *M. fragrans* and *C. annum* after 24 hours of exposure suggest that the plant materials would not give good protection to cowpea seeds. This opinion is further supported by the findings of Onekutu *et al.* (2015) that any natural material which cannot control *C. maculatus* at an economically justified concentration within 24 hours is unlikely good control agent against the insect pest. The modes of action of the test materials are contact and fumigation (Adedire and Lajide, 1999; Asawalam and Emosairue, 2006; Asawalam *et al.*, 2007; Franccedil *et al.*, 2009; Ukeh *et al.*, 2010). *P. nigrum* that most effectively protected against bruchid infestation is edible and thus, poses no risk to human health and the environment, unlike permethrin. Results obtained from this study demonstrate insecticidal potentials of these plant powders against *C. maculatus* in stored cowpea seeds.

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REFERENCES

Adedire, C. and T. Ajayi, 1996. Assessment of the insecticidal properties of some plant extracts as grain protectants against the maize weevil, *Sitophilus zeamais* Motschulsky. Nigerian Journal of Entomology, 13(1): 93-101.

Adedire, C.O. and O.E. Ajayi, 2003. Potential of sandbox, Hura crepitans (L.) seed oil for protection of cowpea seeds from *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)infestation. Journal of Plant Diseases and Protection, 110(6): 602 - 610.

Adedire, C.O. and L. Lajide, 1999. Toxicity and oviposition deterrency of some plants extracts on cowpea storage bruchid, callosobruchus maculatus fabricius. Journal of Plant Diseases and Protection, 106: 647 - 653.

Adedire, C.O., O.M. Obembe, R.O. Akinkurolere and S.O. Oduleye, 2011. Response of callosobruchus maculatus (coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. Journal of Plant Diseases and Protection, 118(2): 75-79. Available at: https://doi.org/10.1007/bf03356385.

Asawalam, E.F. and S.O. Emosairue, 2006. Comparative efficacy of piper guineense (schum and thonn) and pirimiphos methyl as poison against sitophilus zeamais (motsch.). Electronic Journal of Environment, Agriculture and Food Chemistry, 5(5): 1536 - 1545.

Asawalam, E.F., S.O. Emosairue, F. Ekeleme and R.C. Wokocha, 2007. Insecticidal effects of powdered parts of eight nigerian plant species against maize weevil, sitophilus zeamais motschulsky (coleoptera: Curculionidae). Electronic Journal of Environment, Agriculture and Food Chemistry, 6(11): 2526 - 2533.

Ashamo, M., 2007. Evaluation of contact toxicity and fumigant effect of some plant powders against *Sitophilus zeamais* (Motsch.). Proceedings of the Akure-Humboldt Kellong 3rd SAAT Annual Conference: Medicinal plants in Agriculture, The Nig. Exp. pp: 64-67.

Franccedil, T., J.D.P. Michel, S.M. Lambert, F. Ndifor, W.N.A. Vyry, A.Z.P. Henri and M. Chantal, 2009. Comparative essential oils composition and insecticidal effect of different tissues of piper capense l., piper guineense schum. Et thonn., piper nigrum l. And piper umbellatum l. Grown in cameroon. African Journal of Biotechnology, 8(3): 424-431.

Ileke, K., O. Odeyemi and M. Ashamo, 2012. Insecticidal activity of alstonia boonei de wild powder against cowpea bruchid, callosobruchus maculatus (fab.)[coleoptera: Chrysomelidae] in stored cowpea seeds. International Journal of Biology, 4(2): 125-131. Available at: https://doi.org/10.5539/ijb.v4n2p125.

Ileke, K. and M. Oni, 2011. Toxicity of some plant powders to maize weevil, sitophilus zeamais (motschulsky)[coleoptera: Curculiondae] on stored wheat grains (triticum aestivum). Afr. J. Agric. Res, 6(13): 3043-3048.

Khater, H.F., 2012. Prospects of botanical biopesticides in insect pest management. Pharmacologia, 3(12): 641-656. Available at: https://doi.org/10.5567/pharmacologia.2012.641.656.

Lale, N., 2002. Stored-product entomology and acarology in tropical africa mole publications. Maiduguri, Nigeria, 204.

Lale, N.E.S., 1995. A laboratory study of the comparative toxicity of products from three spices to the maize weevil, sitophilus zeamais. Postharvest Biology and Technology, 2: 51 - 64.

Ofuya, T., 1990. Oviposition deterrence and ovicidal properties of some plant powders against callosobruchus maculatus in stored cowpea (vigna unguiculata) seeds. The Journal of Agricultural Science, 115(3): 343-345. Available at: https://doi.org/10.1017/s0021859600075766.

Ofuya, T., 2001. Biology, ecology and control of insect pests of stored food legumes in nigeria. Dave Collins Publications: Akure, Nigeria: pp: 24-58.

Ofuya, T.I. and O.O. Longe, 2006. Fumigant toxicity of some botanical powders to callosobruchus maculatus (f.) (coleoptera: Bruchidae). Proceedings of the 2nd Annual Conference of the Federal University of Technology Akure, pp; 43 - 48.

Okonkwo, E. and W. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by *Callosobruchus maculatus* and *Sitophilus zeamais* in Nigeria. International Journal of Pest Management, 42(3): 143-146. Available at: https://doi.org/10.1080/09670879609371985.

Onekutu, A., L. Nwosu and N. Nnolim, 2015. Effect of seed powder of three pepper species on the bionomics of cowpea bruchid, callosobruchus maculatus fabricius. International Journal of Scientific and Research Publications, 5(5): 1-5.

Raina, A., 1970. *Callosobruchus* spp. infesting stored pulses (grain legumes) in India and comparative study of their biology. Indian Journal of Entomology, 32(4): 303-310.

Somta, C., P. Somta, N. Tomooka, P.-C. Ooi, D. Vaughan and P. Srinives, 2008. Characterization of new sources of mungbean (vigna radiata (l.) wilczek) resistance to bruchids, callosobruchus spp.(coleoptera: Bruchidae). Journal of Stored Products Research, 44(4): 316-321. Available at: https://doi.org/10.1016/j.jspr.2008.04.002.

Sulehrie, M., P. Golob, B. Tran and G. Farrell, 2003. The effect of attributes of vigna spp. On the bionomics of callosobruchus maculatus. Entomologia Experimentalis et Applicata, 106(3): 159-168. Available at: https://doi.org/10.1046/j.1570-7458.2003.00019.x.

Ukeh, D.A., M.A. Birkett, T.J. Bruce, E.J. Allan, J.A. Pickett and A.J. Mordue, 2010. Behavioural responses of the maize weevil, sitophilus zeamais, to host (stored grain) and non host plant volatiles. Pest Management Science: formerly Pesticide Science, 66(1): 44-50. Available at: https://doi.org/10.1002/ps.1828.